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Neural Mechanisms of Classical Conditioning in Mammals

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Neural mechanisms of classical conditioning in mammals

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SUMMARY

Evidence supports the view that 'memory traces' are formed in the hippocampus and in the cerebellum in classical conditioning of discrete behavioural responses. In the hippocampus learning results in long-lasting increases in excitability of pyramidal neurons that resemble the phenomenon of long-term potentiation. Although it plays a role in certain aspects of conditioning, the hippocampus is not necessary for learning and memory of the basic conditioned responses. The cerebellum and its associated brain-stem circuitry, on the other hand, does appear to be essential (necessary and sufficient) for learning and memory of the conditioned response. Evidence to date supports the view that mossy fibre convey conditioned stimulus information and that climbing fibres conveys the critical 'reinforcement' information to the cerebellum and that 'memory traces' appear to be formed in cerebellar cortex and interpositus nucleus.

1. INTRODUCTION

Some years ago we selected classical conditioning of the eyelid closure-nictitating membrane extension response in rabbit (and other discrete behavioural responses for example limb flexion) as a model paradigm to study brain substrates of basic associative learning and memory. These are discrete behavioural responses, elementary skilled movements, learned to deal with aversive events. There is a wealth of data for both humans and other mammals on the behavioural properties of these elementary learned responses (see, for example, Black & Prokasy (1972)). Furthermore, the classical conditioning paradigm permits fine experimental control of the stimuli (conditioned stimulus (CS); unconditioned stimulus (US)) and the responses (conditioned response (CR); unconditioned response (UR)). It is now clear that at least two 'higher' brain systems become massively engaged as a result of associative learning in these paradigms: hippocampal system and cerebellar system. This evidence is reviewed very briefly and new supporting data are presented.

2. HIPPOCAMPUS

Neuronal unit activity in the hippocampus increases markedly within trials early in training. These increases in unit activity in regions CA_1 and CA_3 form a predictive 'model' of the amplitude-timecourse of the learned behavioural response (CR) and happen before the CR in trials, but only under conditions where behavioural learning occurs (Berger & Thompson 1978). This learning-induced hippocampal response is generated largely by pyramidal neurons (Berger *et al.* 1983) (figure 1). Engagement of neuronal activity in the

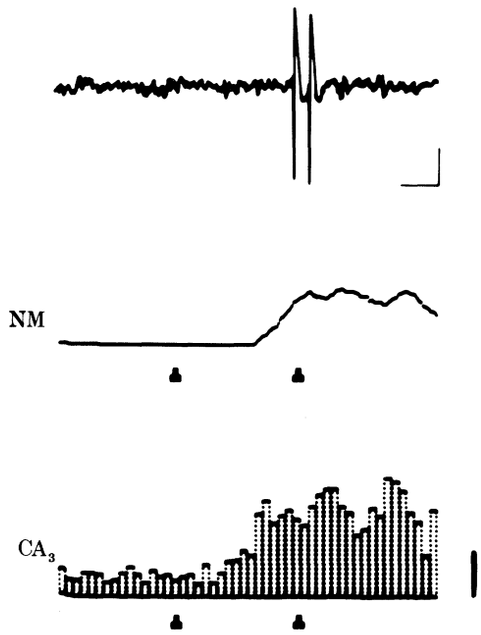

Figure 1. Upper trace: example of individual unit response, identified as a pyramidal neuron in CA_3 of the hippocampus using antidromic activation. (Calibrations: 50 μV and 5 ms). Middle trace: nictitating membrane (NM) extension response averaged over a number of trials (well-trained animal) (trace duration of middle and lower traces: 750 ms; first cursor, tone CS onset; second cursor, corneal airpuff US onset). Lower trace, peristimulus histogram cumulating responses of unit shown in upper trace over the same trials as for the NM response of the middle trace. (Histogram calibration: 25 unit counts per 15 ms time bin). The correlation ratio (η) between the NM and the neuron histogram was 0.78 ($p < 0.01$) (from Berger & Thompson 1978).

hippocampus in learning is of course not limited to classical conditioning, see, for example, the striking 'place' neurons in rodents first described by John O'Keefe. Indeed, hippocampal neuronal activity appears to be influenced by most forms of learning (see O'Keefe & Nadel (1978); Swanson *et al.* (1982) for overviews).

Given such learning-induced (and learning-specific) alterations in neuronal activity in a brain structure, several major issues arise; for example; (i) what are the 'functions' of the structure in learning and memory?; (ii) is persisting neuronal-synaptic plasticity, a 'memory trace', established in that structure (rather than altered activity being relayed there from other structures)?; and (iii) if so, what are the mechanisms underlying the neuronal plasticity?

As far as the hippocampus is concerned the first question is difficult to answer for any form of learning (perhaps the clearest infrahuman lesion results to date are deficits in spatial memory in rodents and delayed non-matching to sample in monkeys). In eyelid conditioning, animals with hippocampal lesions can learn and retain the CR relatively normally; however, such lesions do markedly impair more complex aspects of conditioning, e.g. discrimination reversal, trace learning (see Berger *et al.* (1986) for review). Perhaps in the intact animal the hippocampal system is involved in the formation of experiential or 'declarative' memory, memory about the conditioning situation (the restraining apparatus, tones and corneal airpuffs). Consistent with this is the study by Warrington & Weiskrantz (1973) showing that human amnesic subjects can learn the conditioned eyelid response normally but do not remember the experiences in terms of verbal report.

Several lines of evidence suggest that long-term neuronal plasticity is established in the hippocampus in classical conditioning paradigms. Thus Weisz *et al.* (1984) interpolated single pulse electrical stimulation of the perforant path, recording the monosynaptic population spike response from dentate granule cells, during acquisition of the conditioned eyelid response. There was a marked increase in excitability of this monosynaptic field potential that closely paralleled the development of the learned behavioural response over the days of training. Using a quite different approach, Disterhoft *et al.* trained rabbits in eyelid conditioning and then prepared hippocampal slices. Slices of pyramidal neurons from trained animals showed a marked reduction in the slow after-hyperpolarization compared to neurons in slices from pseudoconditioned control animals (Disterhoft *et al.* 1986).

Learning and long-term potentiation

Note that the basic measure used in Weisz *et al.* (1984), an increase in the monosynaptic granule cell population spike to perforant path stimulation, is a standard measure for assessing long-term potentiation (LTP) in the dentate gyrus. There are several similarities between the enhanced hippocampal pyramidal neuron response in classical conditioning and the enhanced pyramidal neuron response in LTP following tetanus of input pathways. First, both occur with pyramidal neurons (in regions CA_1 and CA_3), secondly, only a small number of stimulations is necessary for each, thirdly, both processes of increased pyramidal neuron response develop in a few minutes, fourthly, the magnitudes of increases are similar in both situations, and finally, there is some similarity in stimulus parameters necessary to establish both forms of plasticity (Swanson *et al.* 1982).

Because of the great current interest in LTP as a putative mechanism of memory storage, relevant studies of the role of LTP in memory are less numerous than one might expect and less conclusive than one might hope. Using a spatial task that requires the hippocampus, Morris *et al.* (1986) reported that administration of an *N*-methyl-D-aspartate (NMDA) receptor antagonist, which blocks LTP, also impairs the subsequent learning of a spatial task, while not affecting a visual one. Ott *et al.* (1982) reported that tetanization of the perforant path improved avoidance behaviour in the shuttle-box, while Berger (1984) agreed that tetanization improves reversal learning of the classically conditioned eye-blink response. McNaughton *et al.* (1986) found that such tetanization impaired acquisition of a spatial maze task.

Induction of LTP in the CA_1 pyramidal cell region of the hippocampus requires activation of NMDA receptors (Collingridge *et al.* 1983), whereas maintenance of LTP is believed to require non-NMDA receptors such as the quisqualate receptor (Kauer *et al.* 1988; Lynch & Baudry 1984; Muller *et al.* 1988; Davies *et al.* 1989). Activation occurs with simultaneous release of glutamate (Collingridge *et al.* 1983) and postsynaptic membrane depolarization (Kelso *et al.* 1986; Malinow & Miller 1986). Since calcium is deemed necessary for the induction of LTP (Lynch & Baudry 1984; Malenka *et al.* 1988), it has been proposed that calcium gains access to the postsynaptic cell through the NMDA channel and thereby activates calcium-dependent processes within the cell that result in increased quisqualate receptor function.

In a collaborative study with Lynch & Baudry (Mamounas *et al.* 1984) we found a significant increase in uptake of [^{3}H]glutamate in hippocampal synaptic membranes in trained animals against unpaired stimulation (CS, US) controls and home cage controls. More recently, with the availability of more specific receptor ligands we have reinvestigated this issue, using [^{3}H]AMPA for the quisqualate receptor subtype and [^{3}H]TCP for the NMDA subtype (Tocco *et al.* 1989). Results were clear, there is significantly more binding of [^{3}H]AMPA to hippocampal tissue (e.g. in the stratum radiation of CA_1) in trained animals than in pseudoconditioned control animals ($p < 0.01$) but no difference in [^{3}H]TCP binding between trained and control animals (see figure 2 for examples). This result is consistent with the general hypothesis that the learning-induced increase in hippocampal neuron activity may share a common mechanism with the process of hippocampal LTP.

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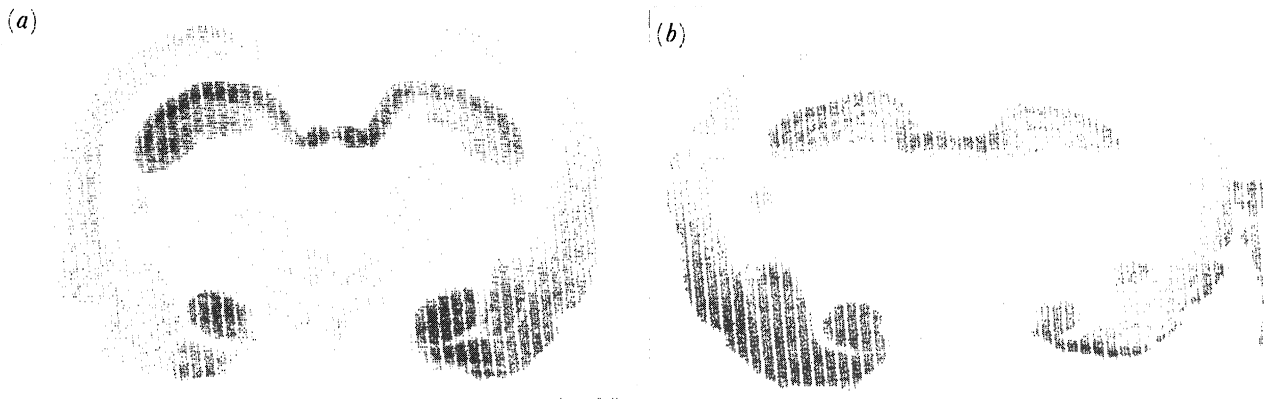

Figure 2. Ligand binding autoradiography generated by incubating 10 μm rabbit coronal sections with 400 nM tritiated AMPA. One can see that the level of binding of the radioactive ligand is clearly increased (darker) in the hippocampus of the conditioned rabbit (*a*), noticeably in the CA_1 region (stratum radiatum), compared to the pseudoconditioned one (*b*), while the level of binding in the cortex is identical. (*a*) Rabbit that has been conditioned for two additional days after reaching criterion. (*b*) Pseudoconditioned control rabbit that has received exactly the same number of stimuli as the animal in *a*.

3. CEREBELLUM

In the course of mapping the brainstem and cerebellum we discovered localized regions of cerebellar cortex and a region in the anterior lateral interpositus nucleus where neuronal activity exhibited the requisite memory trace properties. Patterned changes in neuronal discharge frequency that preceded the behavioural learned response by 60 ms or more (minimum behavioural conditioned response (CR) onset latency is approximately 100 ms), predicted the form of the learned behavioural response (but not the reflex response) and increased over the course of training in close correlation with the development of behavioural learning (McCormick *et al.* 1981, 1984).

We undertook a series of lesion studies: large lesions of lateral cerebellar cortex and nuclei, electrolytic lesions of the lateral interpositus-medial nuclear region, and lesions of the superior cerebellar peduncle ipsilateral to the learned response all abolished the learned response completely and permanently, had no effect on the reflex unconditioned response (UR) and did not prevent or impair learning on the contralateral side of the body (Clark *et al.* 1984; Lavond *et al.* 1981; McCormick *et al.* 1981, 1982*a*, *b*). After our initial papers were published, Yeo *et al.* replicated our basic lesion result for the interpositus nucleus, by using light as well as tone CSs and a periorbital shock US (we had used corneal airpuff US), thus extending the generality of the result (Yeo *et al.* 1985*a*).

Electrolytic aspiration lesions of the cerebellum cause degeneration in the inferior olive: the lesion-abolition of the learned response could be due to olivary degeneration rather than cerebellar damage per se. We made kainic acid lesions of the interpositus. A lesion as small as a cubic millimetre in the lateral anterior interpositus permanently, selectively abolished the learned response with no attendant degeneration in inferior olive (Lavond *et al.* 1985). Additional work suggests that the lesion result holds across CS modalities, skeletal response systems, species, and perhaps with instrumental contingencies as well (Donegan *et al.* 1983; Polenchar *et al.* 1985; Yeo *et al.* 1985*a*).

It is important to stress that the effective lesion in the interpositus completely and permanently abolishes the CR and has no effect on the UR. The anterior lateral region of the interpositus nucleus necessary for learning and memory of the eyelid CR is not much more than a cubic millimetre. If this region is partially but not completely destroyed the CR is markedly and permanently reduced in amplitude and frequency and the onset latency is much increased (Clark *et al.* 1984). Several years ago it was claimed that with extensive overtraining before and after interpositus lesion, very small, long-latency 'CRs' would develop (Welsh *et al.* 1986). However, it is now clear the lesions so reported were incomplete; they did not completely destroy the critical region of the interpositus and did not completely abolish the CR. Indeed, this study constituted simply a replication of the Clark *et al.* (1984) study. To be certain, we replicated their procedures (Thompson *et al.* 1987). Effective lesions completely and permanently abolished the CR and had no effect on the UR regardless of amount of pre- and postoperative training, in agreement with our previous studies and those from other laboratories.

Recently, Welsh & Harvey (1989) have argued that the interpositus lesion result is a 'performance' effect; that the interpositus nucleus is not a part of the essential memory trace circuit but merely modulates the amplitudes of the CR and UR. If this is indeed the case, it is difficult to understand why the interpositus lesion completely and permanently abolishes the CR and has no effect on the UR. To support their argument they measured reflex responses to various test intensities of the US before and after interpositus lesions and reported that the lesion caused a reduction in the amplitude of the UR to low intensity test USs. We have repeated this experiment, measuring UR amplitudes to various intensities of US at several times, beginning before training (Lavond *et al.* 1990). As in earlier studies, the interpositus lesion completely abolished the

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CR and had no effect on the UR (in CS-US paired trials). Furthermore, the lesion had no effect on the URs to test USs, even at the lowest intensity USs. Interestingly, there is a highly significant increase in test UR amplitude as a result of training. We do not yet know whether this increase is due to associative processes or merely to repeated stimulus (CS, US) presentations. In short, there is no evidence to support the claim that the interpositus lesion abolition of the CR is a 'performance' effect.

Recently Lye *et al.* (1988) described the case of a patient they had studied for 69 months following a spontaneous right cerebellar hemisphere infarction. They trained the patient in eyelid conditioning, first on the right eye. Learning was very poor. When training was shifted to the left learning was extremely rapid and robust. They then completed four sequential reversals of the side of training with the same result. These observations constitute a virtually exact replication of the results of our study with rabbits where the interpositus lesion was made before training (Lincoln *et al.* 1982). It is gratifying that basic research on an animal model of associative memory can apply directly to the human condition. This finding in humans increases the likelihood that the mechanisms for this form of memory storage shown in infrahuman mammals will apply closely to such mechanisms in the human brain.

The efferent CR pathway

The essential efferent CR pathway appears to consist of fibres exciting from the interpositus nucleus ipsi-

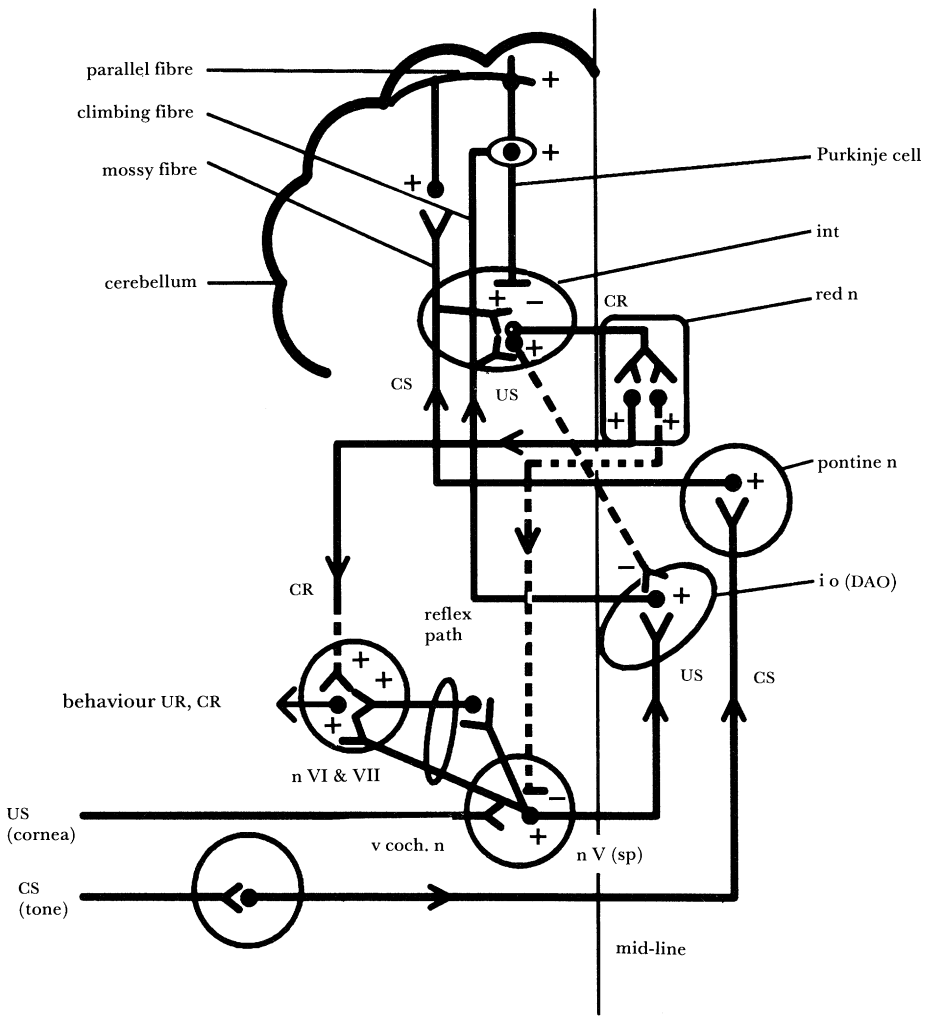

Figure 3. Simplified diagram of hypothetical memory trace circuit for classical conditioning of discrete behavioural responses learned to aversive events. The US (corneal airpuff) pathway consist of somatosensory projections to the dorsal accessory portion of the inferior olive (DAO) and its climbing fibre projections to the cerebellum. The tone CS pathway appears to consist of auditory projections to the cerebellum by way of the pontine nuclei. The efferent (eyelid closure) CR pathway projects from the interpositus nucleus (int) of the cerebellum to the red nucleus (red n.) and via the descending rubral pathway to act ultimately on motor neurons. Evidence to date is most consistent with storage of the memory traces in localized regions of cerebellar cortex and interpositus nucleus. Two descending pathways (dotted lines) are hypothesized to exert inhibitory control from the interpositus nucleus on the DAO-climbing fibres: one is a direct GABAergic pathway from int. to the inferior olivary nucleus (io); the other relays via the red nucleus to act to inhibit projection of somatic sensory information to io (see text for details). Plus symbols indicate excitatory and minuses inhibitory synaptic action. Additional abbreviations : n V (sp), spinal fifth cranial nucleus; n VI, sixth cranial nucleus; n VII, seventh cranial nucleus; V coch n, ventral cochlear nucleus (modified from Thompson 1986).

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lateral to the trained side of the body in the superior cerebellar peduncle, crossing to relay in the contralateral magnocellular division of the red nucleus and crossing back to descend in the rubral pathway to act ultimately on motor neurons (Chapman *et al.* 1985; McCormick *et al.* 1982*b*; Rosenfield *et al.* 1985) (see figure 3). Several lines of evidence argue strongly that the memory trace is localized in structures prior to this CR pathway (Chapman *et al.* 1988).

The CS pathway

Lesion and microstimulation data suggest that the essential CS pathway includes mossy fibre projections to the cerebellum via the pontine nuclei (see figure 3). Thus, sufficiently large lesions of the middle cerebellar peduncle prevent acquisition and immediately abolish retention of the eyelid CR to all modalities of CS (Solomon *et al.* 1986), whereas lesions in the pontine nuclear region can selectively abolish the eyelid CR to an acoustic CS (Steinmetz *et al.* 1987). Consistent with this result is current anatomical evidence from our laboratory for a direct contralateral projection from the ventral cochlear nucleus to this same region of the pons (Thompson *et al.* 1986) and electrophysiological evidence of a 'primary-like' auditory relay nucleus in this poncine region (Logan *et al.* 1986).

Electrical microstimulation of the mossy fibre system serves as a very effective CS, producing rapid learning, on average more rapid than with peripheral CSs, when paired with, for instance, a corneal airpuff US (Steinmetz *et al.* 1986). Finally, appropriate forward pairing of mossy fibre stimulation as a CS and climbing fibre stimulation as a US (see below) yields normal behavioural learning of the response elicited by climbing fibre stimulation (Seinmetz *et al.* 1989). Lesion of the interpositus abolishes both the CR and the UR in this paradigm. All of these results taken together would seem to build an increasingly strong case for localization of the essential memory traces to the cerebellum, particularly in the 'reduced' preparation with stimulation of mossy fibre as the CS and climbing fibre as the US.

The US pathway

Small electrolytic lesions in the rostromedial (face) region of the dorsal accessory olive (DAO) have a most interesting effect on the learned eyelid closure response. Following the lesion (contralateral to the trained eye) the animals showed normal behavioural CRs. But with continued paired training (i.e. tone CS, corneal airpuff US) the CR extinguished in a manner very similar to control animals (with electrodes implanted in the DAO but not lesioned) where the corneal airpuff was discontinued and the animals were given conventional CS only extinction training (McCormick *et al.* 1985). The effective DAO lesion has no effect on the UR to corneal airpuff stimulation. The lesion was made before training in a separate group of animals and they were completely unable to learn the conditioned response.

Yeo *et al.* (1986) replicated our inferior olive lesion study. They also found that the CR was eliminated following inferior olive lesions in previously trained animals. However, they reported that the abolition was immediate, i.e. there was no extinction. There were several procedural differences between the experiments. Thus lesion size may be relevant; our lesions were quite small. More recently we have replicated the experiment by using chemical lesions (Mintz *et al.* 1988). Because climbing fibres projecting to the cerebellum from an olive cross the mid-line and ascend in the vicinity of the contralateral olive, electrolytic lesions of one olive destroy climbing fibre projections from both olives. As expected, NMDA lesions of one olive eliminated CRs of the eyelid contralateral to the lesion but spared ipsilateral CRs. These chemical lesions were larger than our earlier electrolytic lesions and 'extinction' was much more rapid. There was no recovery of the contralateral CR, even after many weeks of postoperative repair and training (learning takes only two to three days in normal animals). Voneida *et al.* (1989) report a prolonged 'extinction' effect of the conditioned forelimb flexion response in cats following lesions of the inferior olive.

Electrical microstimulation of the DAO can evoke a wide range of discrete behavioural responses (eyelid closure, limb flexion or extension, head turn). The nature of the response is determined by the location of the stimulating electrode, consistent with the organization of somatic sensory projections to the DAO (see, for example, Gellman *et al.* 1983). If a movement-evoking DAO stimulus is now used as a US and paired with a tone CS, the animal learns to perform exactly the same behavioural response (phasic movement) as a conditioned response to the tone CS (Mauk *et al.* 1986). The timecourse of learning and the properties of the learned movement appear identical to conditioned responses learned with an aversive peripheral US (e.g. corneal airpuff, paw shock). Interestingly, electrical microstimulation of the DAO that serves as an entirely adequate US is not all aversive to the animal (Mauk *et al.* 1986; Steinmetz *et al.* 1989).

We argue that the effective stimulus to obtain this result is to the cells of origin of climbing fibre projecting to the cerebellum from the DAO. There are, of course, alternative explanations, mostly relating to the possibility of stimulation of fibres of passage. We have completed a number of control procedures and observations in an attempt to rule out these alternatives (see Steinmetz *et al.* (1989); Thompson (1989)). Perhaps the strongest lines of evidence are: (i) the effective DAO stimulus evokes a characteristic climbing fibre field potential in cerebellar cortex; (ii) interpositus lesions abolish *both* the CR and the DAO elicited UR; and (iii) movements elicited by stimulation in the reticular formation adjacent to the DAO cannot be trained to neutral stimuli.

In current work (Shinkman *et al.* 1989; Swain *et al.* 1989) we find similar result when stimulating white matter underlying cerebellar cortex as a US (such stimulation evokes movements), thus replicating a classic but forgotten study by Brogden & Gantt (1942). Interpositus lesions abolish both the CR and UR, suggesting that the movement-eliciting circuit relays

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through the interpositus rather than via antidromic activation of reflex afferents. We suggest that the effective US is activation of climbing fibres, but mossy fibre and Purkinje axons are of course also activated.

Purkinje neuron responses

The evidence summarized above led us to propose the hypothetical essential (necessary and sufficient) memory trace circuit shown in figure 3. This diagram is consistent with and supported by all available empirical data, which constitute a striking validation of classical theories of the role of the cerebellum in motor learning (Albus 1971; Eccles 1977; Ito 1972; Marr 1969). Our hypothesis makes a very clear prediction regarding Purkinje cell activity. Simple spikes activated by mossy-parallel fibres constitute the vast majority of Purkinje cell responses. Since Purkinje neurons inhibit interpositus neurons and learning involves massive increases in interpositus neuron activity in the CS period, Purkinje neuron simple spikes ought to decrease in frequency. This is precisely what we have found (preliminary reports in Donegan *et al.* (1985); Foy & Thompson (1986)) in recordings mostly from lobule H VI of the cerebellar cortex ipsilateral to the trained eye made both before and after training. We studied simple spike activity in response to tone stimuli in 118 Purkinje neurons in untrained animals. In this sample, 63% showed clear changes in simple spike discharge frequency following tone onset. Of these, 68% showed increases in simple spike frequency and 32% showed decreases. We recorded from 77 Purkinje neurons in the training animal (tone CS). In this sample, 87% showed changes following tone onset. Of these, 69% showed decreases in simple spike frequency and 31% showed increases. Thus training resulted in a marked increases in the percent of Purkinje neurons showing a decrease in simple spike frequency in the CS period. An example is shown in figure 4. This result is at least consistent with Ito's (1984) hypothesis of long-term-depression as a mechanism of synaptic plasticity in the cerebellum. In current work in collaboration with Greenough, we have anatomical evidence suggesting a decrease in parallel fibre synapses on Purkinje neuron distal dendrites as a result of training (Anderson *et al.* 1989), which is nicely consistent with the neurophysiological data just described.

Complex spikes are evoked in Purkinje neurons by climbing fibre activation (one climbing fibre synapses on a given Purkinje neuron and exerts a powerful excitatory action; upwards of 200000 parallel fibres synapse on a given Purkinje neuron, each synapse exerting a weak excitatory action). The spontaneous rate of complex spikes in a Purkinje neuron in the absence of stimulation is low, perhaps 2–4 s^{-1} in the cerebellum of the awake rabbit. We examined the likelihood that corneal airpuff US onset would evoke a complex spike. In untrained animals, of 88 Purkinje neurons that showed simple spike changes to tone, some 61% showed US onset evoked complex spikes. In well-trained animals, of 68 Purkinje neurons that showed changes in simple spike frequency in the CS period, only 27% showed US onset evoked complex spikes. This low incidence of US evoked complex spikes in the trained animal has also been reported by Berthier & Moore (1986).

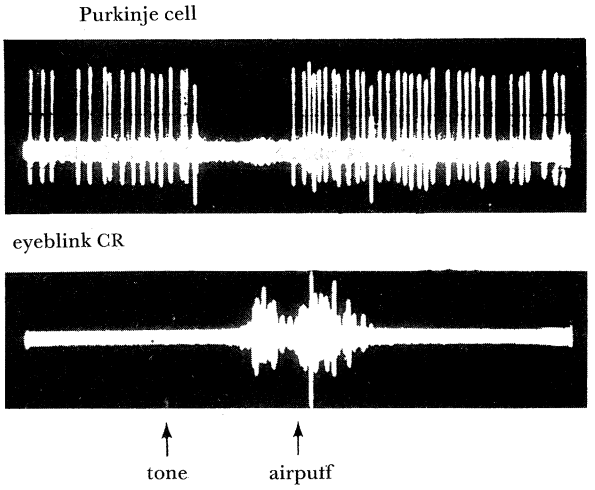

Figure 4. Upper trace: Individual example of identified Purkinje neuron response in a paired CS-US trial in a well-trained animal. First arrow, tone CS onset; second arrow, corneal airpuff US onset; time between onsets 250 ms. Lower trace: Behavioural eyelid closure response (EMG of obicularis oculi). Note that simple spike discharges of the Purkinje neuron shut down approximately 90 ms before onset of the behavioural CR and 25 ms after tone CS onset in this example.

The locus of the memory trace

Lesion studies summarized above indicate that the neuronal circuit necessary for learning and memory of the conditioned eyelid response (and other discrete behavioural responses learned with aversive USs) includes the inferior olive-climbing fibres, pontine nuclei and middle cerebellar peduncle (mossy-fibres), cerebellum, superior cerebellar peduncle, red nucleus and descending rubral pathways, as well, of course, as the sensory afferents for the CS and US and the motor nuclei necessary to generate the behaviour. The lesion method can define a necessary circuit but cannot, by itself, localize a memory trace.

Electrical stimulation can be helpful in identifying the sufficient circuit and possible loci of the memory trace. Thus, stimulation of the appropriate region of the red nucleus elicits eyelid closure but such a response-evoking stimulus is completely ineffective as a US to yield learning of the response to a neutral stimulus. Similarly, stimulation of interpositus is ineffective as a US. However, after paired tone-interpositus stimulation, subsequent learning to tone-corneal airpuff shows substantial savings. Stimulation of cerebellar white matter, on the other hand, is an effective US, as is stimulation of the DAO climbing-fibre system. Other than reflex afferents, I know of no other places in the brain where stimulation that evokes a specific skeletal muscle response can serve as an effective US to train that response to a neutral stimulus. At this stage the most economical hypothesis is that activation of climbing fibres is the critical reinforcing event.

Electrical recording can provide considerable com-

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plimentary evidence both for identification of the memory circuitry and for localization of the trace. As noted earlier, the learning-induced increase in neuronal response in the interpositus is highly predictive of the occurrence and form of the behavioural CR. It is isomorphic with the behavioural CR except in having a shorter onset latency trials. This, together with evidence reviewed above, suggests strongly that the memory trace is localized to the interpositus nucleus or to systems afferent to the interpositus for which the interpositus is a mandatory efferent, perhaps to both. The fact that electrical stimulation of axons in the middle cerebellar peduncle (that form mossy fibre in the cerebellum) is a very effective CS suggests that the memory trace is formed in the cerebellum, as does the fact that appropriate stimulation of such fibre as a CS, and of DAO-climbing fibre as a US results in normal behavioural learning. Preliminary neuronal unit recordings in the region of the pontine nuclei that appears to be the necessary relay for auditory CS activation of the cerebellum indicate no learning-related activity. Indeed, the earliest neuronal sign of learning-induced plasticity yet observed in training trials is in Purkinje neurons in cerebellar cortex (see figure 4).

Yeo *et al.* (1985*b*) reported that lesions removing only the cerebellar cortex of lobule H VI abolished the CR. We have been unable to obtain this result. However, we concur that H VI and particularly larger extents of cerebellar cortical removal that include H VI can markedly impair and even temporarily abolish the CR (Lavond *et al.* 1987). Indeed, we now have several cases of very large cerebellar cortical ablations that prevented learning (Logan *et al.* 1989). Our disagreement is really only one of degree. It is a difficult problem experimentally to remove completely the tissue of H VI without damaging the interpositus. I have always hoped that the essential memory traces are formed in cerebellar cortex; there is vastly more neuronal machinery in the cerebellar cortex than in the interpositus nucleus. The organization of somatosensory projections to cerebellar cortex favours the possibility of multiple cortical sites of storage (Shambes *et al.* 1978). Wally Welker (personal communication) stresses that there are many separate representations of the body surface in cerebellar cortex (as many as 20?), each with a seemingly disjoined 'fractured' somatotopic organization. Our Purkinje neuron recordings support the view that memory traces, essential or not, are formed in cerebellar cortex. The onset latencies within trials of learning-induced changes in simple spike discharge frequency of Purkinje cells are on average shorter than those of interpositus neurons and are the earliest neuronal sign of learning as yet observed following CS onset, as noted above. As far as the essential memory trace is concerned the hypothesis most consistent with all the available evidence is that it is formed in cerebellar cortex, in multiple sites, and in the interpositus nucleus.

Relation to theories of classical conditioning

In current work we are attempting to relate the empirical results reviewed above characterizing the cerebellar–brain stem circuitry essential for classical conditioning of discrete responses, and our hypothetical memory circuit (figure 3), to theoretical formulations of learning and memory (Donegan *et al.* 1989; Thompson 1989). Thus among the most influential mathematical theories of classical conditioning is that developed by Rescorla & Wagner (1972). For learning, i.e. excitatory conditioning, they postulated that the increase in associative strength between CSs and a given US, ΔV_i, is given by:

$$\Delta V_i = \beta(\lambda - \sum_s V_s),$$

where β is a rate parameter, λ is the maximum associative strength conditionable with a given US and $\Sigma_s V_s$ is the sum of associative strengths between all CS elements present on the trial and the US. The heart of this formulation is the 'error correcting' algorithm, which states that the greatest increments in associative strength occur with the greatest discrepancy between current associative strength ($\Sigma_s V_s$) and maximum possible strength (λ), namely before any learning has occurred. When learning has reached asymptote (λ), no additional associative strength will accrue with additional training. As we and others have noted, this is an instance of the least mean squares algorithm in adaptive filter theory and is equivalent to the 'delta rule', a widely used error correcting algorithm in connectionist learning theory (Rumelheart & McClelland 1986; Widrow & Hoff 1960). Among other accomplishments the Rescorla-Wagner theory provides a straightforward explanation of the blocking effect (Kamin 1969), thought by some to be an instance of a cognitive phenomenon in classical conditioning.

As noted earlier, several lines of evidence support the view that the inferior olive DAO-climbing fibre system is the essential US or reinforcing system in classical conditioning of discrete behavioural responses. More specifically, we hypothesize that the reinforcement strength of the US on a given trial is determined by the proportion of climbing fibre activated by the US on that trial relative to the total number activated by the US before training. The fact that the US commonly evokes complex spikes in Purkinje neurons before training but rarely does so in the well trained animal (see above) is supportive of this hypothesis.

Recent anatomical and physiological data provide possible mechanisms whereby the cerebellar-brain stem circuit could produce this result. Thus electrical stimulation of the red nucleus results in inhibition of the projection of somatosensory information from relay nuclei to the inferior olive, the inhibition presumably occurring in the somatosensory relay nuclei (Weiss *et al.* 1985). In addition there is a direct projection of GABAergic (inhibitory) neurons from the interpositus nucleus to the inferior olive (Andersson *et al.* 1987; Nelson and Mugnoini 1987). As noted earlier, over the course of training there is a massive increase in neuronal activity in the appropriate region of the

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interpositus in the CS period, *preceding* the onset of the US. Assuming this increase reflects increases in activation of both the red nucleus and inferior olive projecting neurons, there will be a marked increase in inhibition of US evoked DAO-climbing fibre activation of cerebellum over the course of learning, thus yielding a decrease in US evoked complex spikes in Purkinje neurons. The strong prediction is that as learning develops, US activation of the DAO should shut down. In current work we are recording unit activity from neurons in the DAO over the course of training. Before training, US onset evokes a marked neuronal response in inferior olive neurons in the appropriate region of the DAO. Over the course of training this US evoked response decreases and virtually ceases on paired trials when CRs occur, but is still present in original form on US alone test trials, as predicted.

In short, evidence to date supports the hypothesis that climbing fibres convey the critical 'reinforcement' input for memory trace formation in the cerebellum in classical conditioning of discrete responses. Further, the organization of the cerebellar-brain stem circuit, namely, the descending inhibitory pathways from the interpositus, can provide a neuronal 'instantiation' of the error-correcting algorithm in the Rescorla–Wagner theory and certain other quantitative theories of learning. Indeed, our hypothesized circuitry is in close accord with Wagner's (1981), 'sometimes opponent process' theory. Perhaps most intriguing is that the error correcting algorithm in the neuronal circuit is an emergent property of the organization of the circuit itself; no special synaptic-molecular processes need be invoked.

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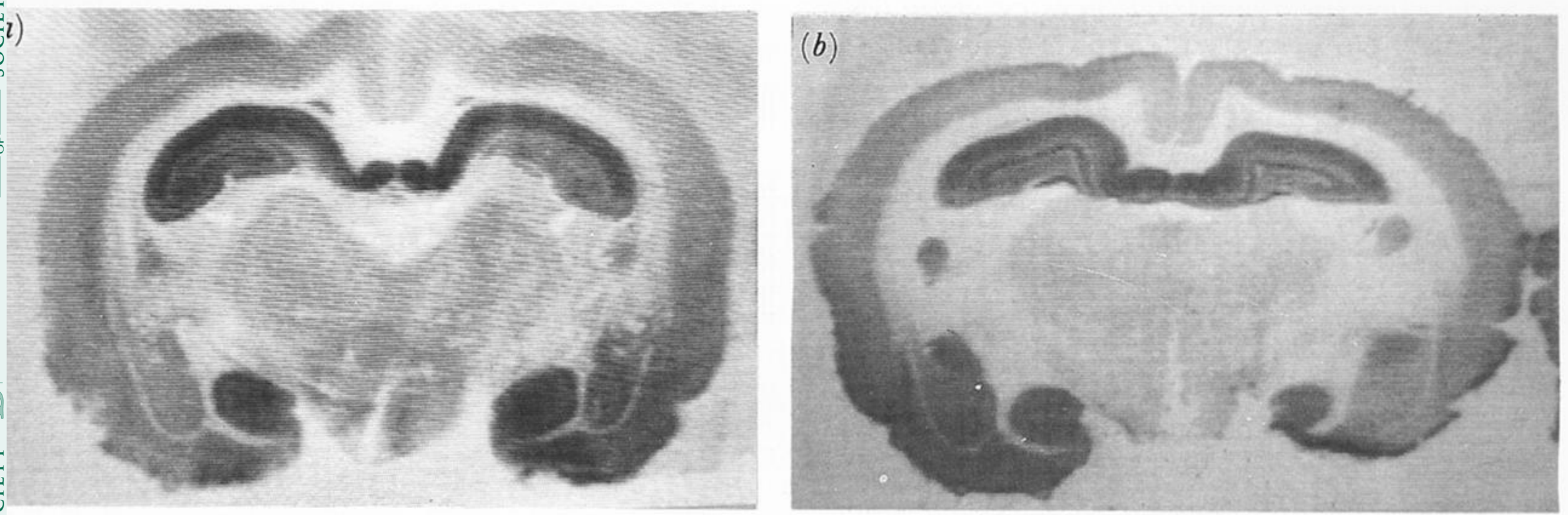
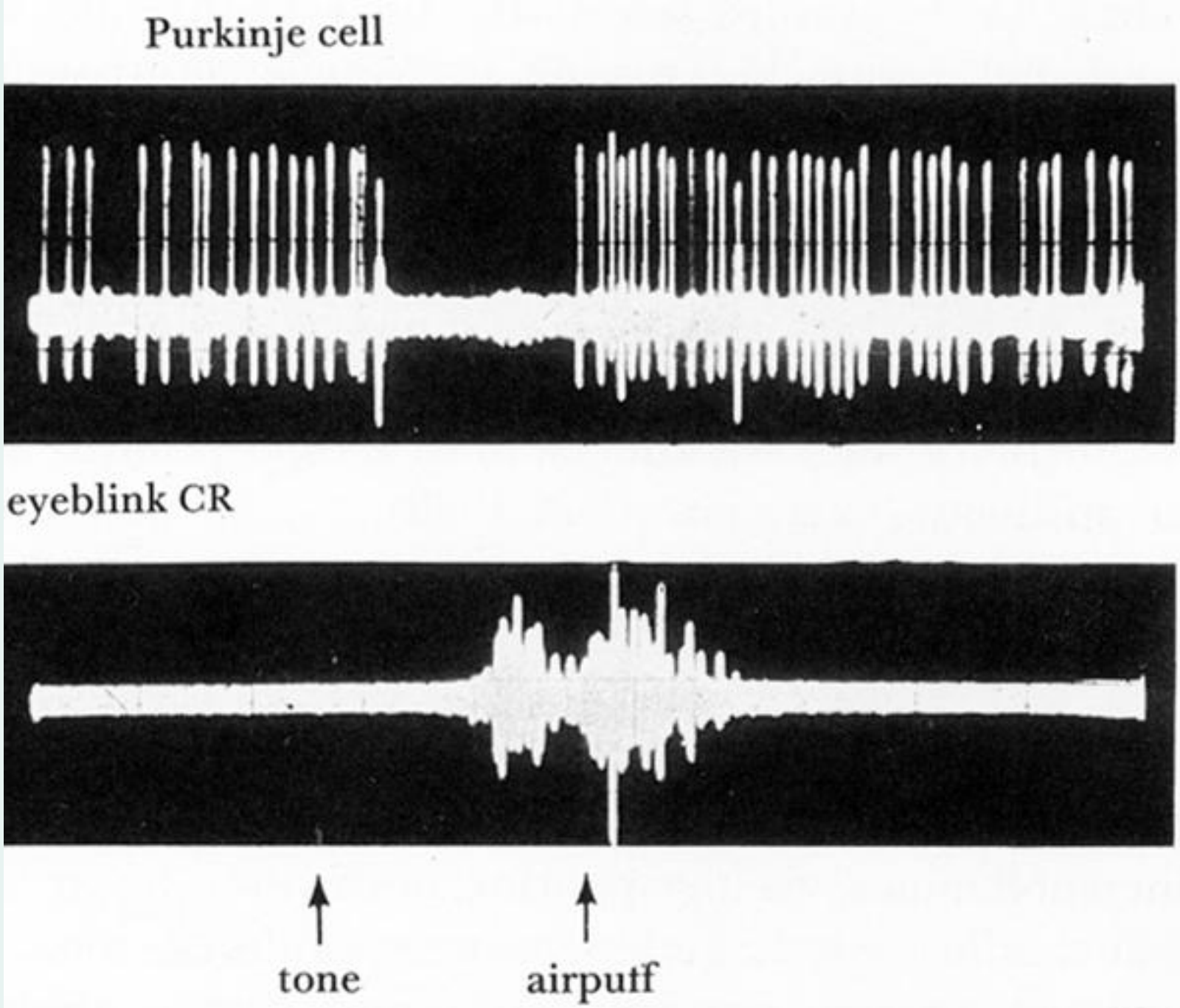


Figure 2. Ligand binding autoradiography generated by incubating 10 μm rabbit coronal sections with 400 nM tritiated AMPA. One can see that the level of binding of the radioactive ligand is clearly increased (darker) in the hippocampus of the conditioned rabbit (*a*), noticeably in the CA_1 region (stratum radiatum), compared to the pseudoconditioned one (*b*), while the level of binding in the cortex is identical. (*a*) Rabbit that has been conditioned for two additional days after reaching criterion. (*b*) Pseudoconditioned control rabbit that has received exactly the same number of stimuli as the animal in *a*.



gure 4. Upper trace: Individual example of identified
irkinje neuron response in a paired CS-US trial in a well-
ained animal. First arrow, tone CS onset; second arrow,
rneal airpuff US onset; time between onsets 250 ms. Lower
ace: Behavioural eyelid closure response (EMG of obicularis
uli). Note that simple spike discharges of the Purkinje
uron shut down approximately 90 ms before onset of the
havioural CR and 25 ms after tone CS onset in this example.

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